SEA GRANT PUBLICATION 48

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UNIVERSITY OF CALIFORNIA
SEA GRANT COLLEGE PROGRAM

IMR REFERENCE 76–6

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MAIL CODE A-032
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IMR Reference 76-6
Sea Grant Publication No. 48
May 1976

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APPROVED FOR DISTRIBUTION:
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INTRODUCTION

During the past five years the pace of research on the controlled culture of American lobster has markedly increased. The individual contributions of several authors have enhanced the potential to rear lobster larvae to marketable size under controlled conditions. We have attempted here to review the works of several investigators and relate their findings to one phase of the controlled rearing process. This report describes aspects of culture pertinent to the production and culture of larval lobsters. These include the acquisition of ovigerous females, conditioning of broodstock, collection of larvae, feeding, disease control, and physical systems for larval culture.

Obtaining Ovigerous Females

There are three means of acquiring ovigerous females. The simplest is to obtain females carrying eggs from fishermen under license to trap berried females for this purpose. The major advantage of this method is the ability to select females in various stages of egg development that do not require a great deal of time and holding space. The disadvantage is the limited hatching period coincident with the natural ambient temperature cycle although some manipulation is possible with temperature control.

Another means of producing an ovigerous stock is to obtain 0.8 kilogram or larger non-ovigerous females in August or September. Sixty to eighty percent of these females will extrude eggs in the next one to seven months. The variation in time of extrusion is the result of the interplay between the state of development of the ovary at the time of capture and the temperature at which the female is held subsequent to capture. The advantages

of this method are the ability to obtain larvae producing animals without special permit (non-ovigerous females are not restricted in the commercial fishery) and the greater flexibility in manipulation of hatching date due to the fact that a greater part of the reproductive process can be extended or compressed as desired.

The third method of obtaining ovigerous females is to completely contain the reproductive process in the laboratory. Newly molted females are mated, extrude, and finally, hatch in captivity. This is accomplished at the Massachusetts State Lobster Hatchery and recently at the Bodega Marine Laboratory. Unfortunately, the cycle is long and poorly understood. Fertilization may not take place, eggs may be lost at extrusion or the viability of larvae is low. Although the reproductive process has occurred entirely in controlled condition, it is not always successful. The greater success in an ambient temperature regime at the Martha's Vineyard Hatchery indicates temperature dependence of the process. It is possible that the normal 18 month cycle may be reduced with a compressed temperature regime mimicing the natural fluctuation. Photoperiod may also have a regulatory effect. The nutrition of the female and its relationship to its reproductive condition is not understood. The containment of the life cycle is a relatively expensive process. The two to three years required to attain sexual maturity plus additional time for egg development and the need to maintain large numbers of adults for extended periods make total broodstock control a lengthy and involved process. These drawbacks are compensated for, in part, by the potential to produce larvae on a year-round basis and the capability to do selective breeding. This method also allows the production of larvae with a known genetic constituency which can reduce the natural variability.

Regardless of the method used to obtain ovigerous females it is of the utmost importance to use care when the rearing facility is located outside the natural lobster territory. Homarus is vulnerable to the systemic pathogen Aerobacter virulens (gaffkemia, redtail) which can be transmitted to the spiny lobster, Panulirus interuptus, and other crustaceans. It is suggested (Kellog, et al., 1974) that exotic animals be quarantined for 2 weeks while effluent waters are treated with chlorine. The disease should kill any infected lobster in this time at 20°C (Stewart, 1969).

Fecundity and Egg Development

Saila, Flowers and Hughes (1969) derived an equation relating the size of the female with her fecundity by means of sampling oviposited females and calculating the regression equation as follows:

$$\log Y = -1.6017 \times 2.8647 \log X$$

where

Y = fecundity (number of eggs)

X = size (carapace length in mm).

For example, an 85 mm female is calculated to have Y eggs by the following equation:

$$\log Y = -1.6017 \times 2.8647 \log 85$$

$$\log Y = -1.6017 \times 2.8647 \ (1.9294)$$

$$Y = 8424 \text{ eggs}$$

Lobsters in the wild hatch from approximately April until August with the peak activity occurring in late June. A more extended hatching period is possible if broodstock are acclimated and held at temperature extremes. Perkins (1972) described the effect of temperature on lobster embryos and developed an index of development that may be used to predict accurately the hatching date of eggs held between 7°C and 25°C. The index Perkins

used was the length plus the width of the eye spot, measured in μm, divided by 2. When the index approaches 560 the egg is ready to hatch. The time from extrusion to hatching is obtained by the formula:

$$\frac{A_1}{X_1} = \frac{A_2}{X_2}$$

where A_1 is the time (in weeks) from eye pigment appearance to hatch at 20°C, A_2 the observed time from extrusion to hatch at 20°C, X_1 was the observed time from eye spot appearance to hatching at a given temperature, and X_2 is the time from extrusion to hatch at the same given temperature. If time from extrusion to the onset of eye pigmentation is not known, the time to hatch may be found by subtracting the given eye index from 560 and dividing by the value obtained from the following equation:

$$Y = -8.3151 + 2.6019(X)$$

where Y is the increase in eye index per week and X is the temperature.

For example, given a female lobster to be held at 20°C with eggs showing an average eye index of 360 microns, how long will it take to hatch the eggs.

$$\begin{array}{r} 560 \\ -360 \\ \hline 200 \end{array}$$

$$Y = -8.3151 + 2.6019(20)$$

$$= 43.7229$$

$$\text{Time to hatch} = \frac{200}{43.7} = 4.6 \text{ weeks}$$

We have found Perkins' method to be a practical means of predicting the hatch date. A high degree of flexibility is attainable when scheduling hatching dates using the temperature control technique, making it theoretically possible to maintain larval culture on a year-round basis. Larvae

have been hatched at the Bodega Marine Laboratory in every month from October to August and at Martha's Vineyard, the year around. It is appropriate to mention that temperature control of the developing embryo is a delicate process. Acclimatization must take place in gradual increments of no more than 3°C per day. Eggs subjected to the stress of immediate transfer between 5°C and 25°C will probably die, although the female will often make the transition. Experience has also shown that eggs taken to one extreme and exposed to fluctuating temperatures will produce larvae of poor quality and reduced viability.

Developing eggs have been found to be susceptible to at least two disease conditions that may occur simultaneously. Epibiotic growth on the egg casing is most often filamentous micro-organisms including Leucothrix mucor. L. mucor was first reported on crustacea by Johnson, et al. (1971). Other bacteria, cyanophytes, diatoms and vorticellids have also been observed (Nilson et al., 1976a). These epibionts are thought to restrict metabolic exchange with the environment. The pathogenic fungus Lagenidium sp. has also been found on lobster eggs (Nilson, et al., 1976b). This fungus invades the tissues of the embryo. These diseases are easily detected as described by Fisher, et al. (1976a). Examination of eggs prior to hatch will indicate the type and extent of disease problems. Badly diseased egg masses may be rejected without further loss of time and energy. The only successful treatment yet found for these conditions on lobster eggs, and then only lightly infested ones, is dipping the ovigerous female in a 5 ppm solution of malachite green for 10 minutes (Fisher, et al., 1976b). This inhibits the growth of the disease agents apparently without harm to the female or the eggs.

Larval Rearing

Upon hatching, the lobster larva is described as being in the first of four larval stages. The hatching female is usually acclimated to the larval rearing temperature when hatching is near. This is to avoid a large temperature difference for the new larvae transferred to the larval rearing kreisel. The larvae are free swimming and are carried by a gentle current into a screen basket. The basket can be removed and the larvae carried to the rearing kreisel where they are rinsed off into the vessel. The larval rearing process is relatively simple compared to the rearing of other crustacea. The newly hatched animal is capable of eating live adult brine shrimp and a variety of other foods including frozen brine shrimp, daphnia, squid, fish, finely chopped clam, abalone, live plankton, and artificial preparations. In all tests, live foods were taken more readily than frozen. Live adult brine shrimp, if available, are superior since they are alive until eaten. They do not greatly contribute to the bacterial fouling of tank surfaces and have good dispersion characteristics.

The objective of the kreisel is to disperse the larvae. If the larvae merge in groups, large mortalities will result from cannibalism. When food and larvae are uniformly dispersed and proper densities of both are maintained, larval survival is excellent. Hughes, Shleser and Tchobanoglous (1974) described the hydraulic characteristics of the circulation device and the companion vessel where the larvae and food are constantly stirred in a spiral upwelling pattern. The maximum density that the 40 liter kreisel can successfully contain is about 2,500 larvae although maximum survival is attained with around 1,700 larvae. If live adult brine shrimp are the food source, they should be kept in a proportion of approximately 4 brine

shrimp to 1 larva. This proportion may be ascertained by counting aliquots from the culture vessel. The constant availability of food reduces cannibalism to a minimum. If other foods are used, about 10 ml of the food should be added every three hours. As the larvae develop to the second, third, and fourth stages, feeding increases and food must be available as required.

Larvae may be fed heavily late at night and then not until morning. Serfling et al. (1974a), however, devised a mechanical feeder that adds a portion of live or frozen brine shrimp into the kreisel at timed intervals during the night. This device may be desirable if 70% or greater survival, or reduced labor costs are an objective.

Hughes and Matthiessen (1962) described the effect of temperature on the development of H. americanus larvae. At 22°C larvae reached the fourth stage in as little as 9 days while those cultured at 15°C took as long as 35 days. Various constantly controlled temperatures have produced similar results at the Bodega Marine Laboratory. Larvae will also suffer if the temperature fluctuates erratically. Gradual adjustment of the temperature by no more than 3°C per day to the desired level is preferable to larger daily fluctuations.

A variety of filamentous epiphytes are known to contaminate lobster larvae (Nilson, et al., 1976a). The most thoroughly studied of these epiphytes is the filamentous marine bacterium Leucothrix mucor. Infestation with L. mucor has caused heavy mortalities in some batches of larvae. Generally, the earlier the onset of infestation, the more severe the losses. The filamentous growth on external surfaces hinders the animal as it attempts to feed or molt and may restrict respiration. The long dense filaments of the bacteria entangle larvae, food, and exuvia into mats. Maintenance

of a 1 mg/l concentration of streptomycin sulfate in the larval system has been used to prevent this disease and is reported to reduce mortalities (Serfling, personal communication). The use of antibiotics, however, is often undesirable due to the incompatibility with biological filtration, the difficulty in maintaining therapeutic levels, and the possibility of enhancing the growth of fungi and resistant strains of bacteria.

A disease that is also carried over from the embryo is the fungus Lagenidium (Nilson et al., 1976b). Treatment with streptomycin sulfate to counteract filamentous contamination has been observed to select out all competition and allow the Lagenidium to run rampant through the rearing system. The disease is capable to destroying greater than 90% of the exposed animals in less than 3 days under such conditions.

The hyphae of the Lagenidium permeate the tissues of the larvae. As the infection progresses, germ tubes will protrude from the hyphae and penetrate the integument. At the tip of these germ tubes, germinal vesicles are formed which contain zoospores. These zoospores are released into the seawater environment where they are capable of infecting a new host.

Both the epiphytic and the fungal diseases are restricted to larval stages. Although some hold over cases have been observed in young juveniles, the animals apparently become strong enough to ward off lethal infestation. For this reason, larvae infected near the end of the larval stages will have better survivorship than those infected earlier.

Prophylaxis and treatment for both of these diseases in larval rearing systems has been described by Fisher et al. (1976b). The ovigerous female is dipped in a tank of 5 ppm malachite green solution in sea water for 10 minutes prior to entering the hatching tanks. Upon hatching and semi-daily thereafter, the larvae are dipped in the same concentration for 2 min.

Toxicity levels of malachite green on larval lobsters have been described by Fisher, et al. (1976c) using this method of semi-daily dipping. The dipping procedure inhibits the growth of the pathogens on the surfaces of the animal. The use of ultra-violet irradiation on line in the semi-closed larval rearing system greatly reduces the spread of disease from kreisel to kreisel. The malachite green treatment may be suspended when the animals reach juvenile stages, although U.V. irradiated sea water is still important.

Chitinolytic bacteria are held responsible for another disease found in lobster larvae (Fisher, et al., 1976d), causing erosion and marring of the exoskeleton. This is similar to shell disease of adult lobsters and may be caused by the same organisms. It has been found that young juveniles show increased susceptibility to this disease when under conditions of stress, especially dietary insufficiencies. Although this disease is infrequently found in larval rearing systems, it is potentially more detrimental to the thin exoskeletons of the larvae than to adult lobsters. It has been observed to kill greater than 75% of the animals in a larval system.

Another disease caused by the fungus Haliphthoros milfordensis (Fisher, et al., 1975) is normally restricted to young juveniles, however, cases have been observed in 3rd and 4th stage lobsters. No treatment is yet recommended for this disease, although losses have been found to be greatly reduced with thorough cleaning. The extent of this disease is minimal in the larval stages, but greater losses occur in the young juveniles.

The most important aspect of disease control is the maintenance of the system. The larval rearing kreisels should be kept free of food remnants, exuvia, and dead animals that harbor these disease agents. The filters (see Rearing Systems) are backflushed 2-3 times per week or if the system is closed and dependent on biological filtration the bed should be well

conditioned, not overloaded, or allowed to become anaerobic. All handling implements are stored in an antiseptic (20 ppm malachite green) solution and should be used for only one system to prevent the spread of disease agents. The animals themselves should be handled with the utmost care to prevent stress that makes them more susceptible to disease. It has been observed that animals that were never exposed to air and handled only minimally showed excellent survival. Handling implements should be designed to maintain the larvae in sea water at all times. Before a system is used it should be chlorinated (10 ppm) and then flushed with fresh water 2 or 3 times before sea water is added, especially if the system previously held diseased animals. The sea water should be filtered and passed through ultra-violet irradiation prior to entering the system. Larval rearing systems should be maintained on a separate system from other stock to prevent contamination from these sources.

Larval Rearing System

A number of different system configurations have been used to rear larval lobsters. They all utilize the spherical bottom kreisel developed by John Hughes and John Sullivan at the Massachusetts State Lobster Hatchery (Figs. 1-3). The tank and circulator have been slightly refined and the details of the construction and operation of the kreisel are described by Hughes, et al. (1974). Regardless of the water system associated with the kreisel, it accomplishes the objectives of (1) dispersing larvae and food uniformly in the water mass, (2) flushing the tank surface clean of particulate waste, and (3) providing a physical environment that does not mechanically damage the larvae.

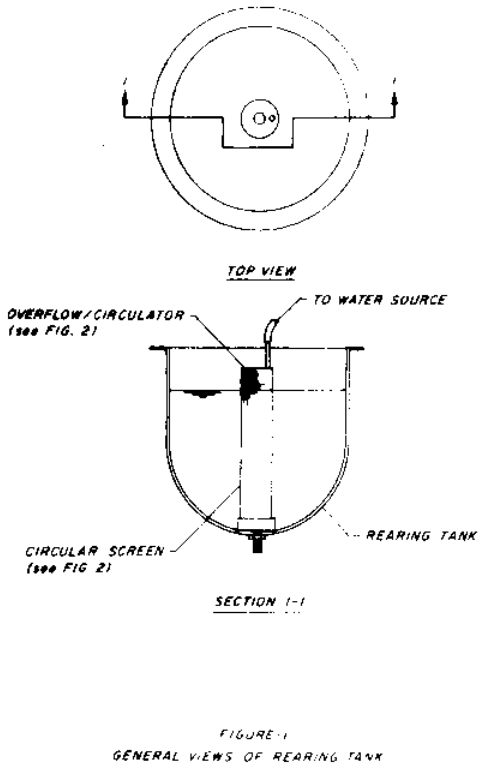

FIGURE 1
GENERAL VIEWS OF REARING TANK

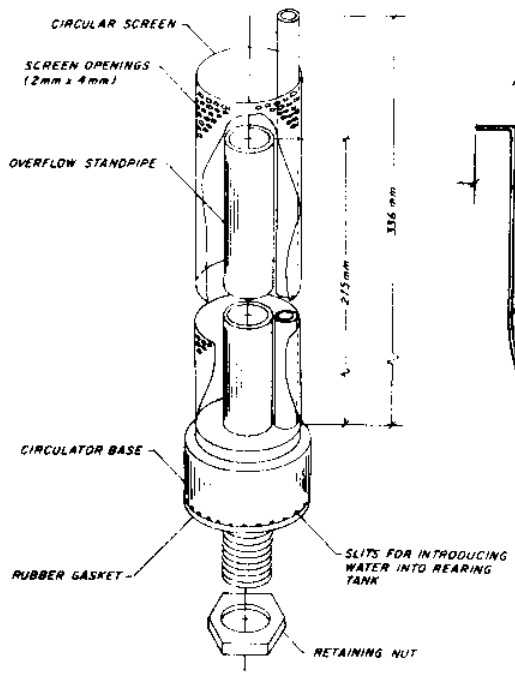

FIGURE 2
PICTORIAL VIEW OF OVERFLOW/CIRCULATOR

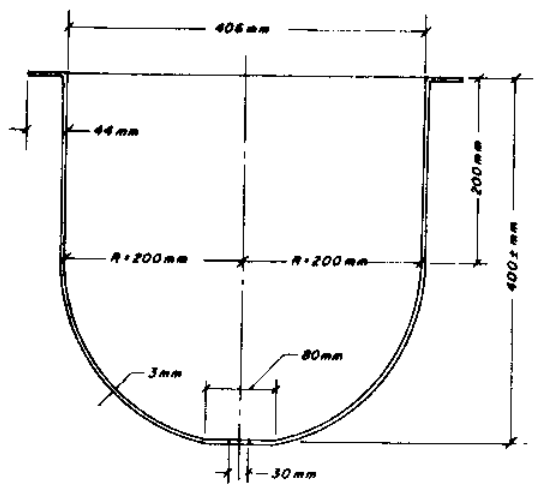

FIGURE 3
REARING TANK CONSTRUCTION DETAILS

Under different operational constraints investigators at the Bodega Marine Laboratory, the Massachusetts State Lobster Hatchery and the California State University at San Diego have developed water systems for larval rearing that fall into three general categories: (1) open, (2) semi-closed, and (3) closed. To operate effectively all three types of systems must provide the following conditions:

1. A continuous reliable supply (4-6 liters/min/kreisel) of sea water with salinity stable between 25 and 35 ppt,
2. Sea water free of pathogenic organisms or a means of excluding them from the rearing system,
3. Sea water near the optimal temperature for the development of the larvae (20°C±3°C) and stability of the temperature regime (±2°C daily),

4. Sea water with low levels of waste metabolites, particularly ammonia. No particular chronic toxic level of ammonia has been established but deleterious effects at levels above 1 ppm NH_3 have been observed.

Open Systems

In an open system, water is supplied to the kreisels on a single pass basis. The flow is regulated by a valve at each kreisel. The overflow is caught by the central standpipe and falls into a trough below leading to a drain.

Incoming sea water will likely require some pretreatment before use. A sand and anthracite filter (Fig. 4) or similar pressure filter can be used to remove silt and organic matter as well as larval invertebrates that will attach and grow within the system. If not removed before use, this material will clog valves and circulator ports and require frequent cleaning.

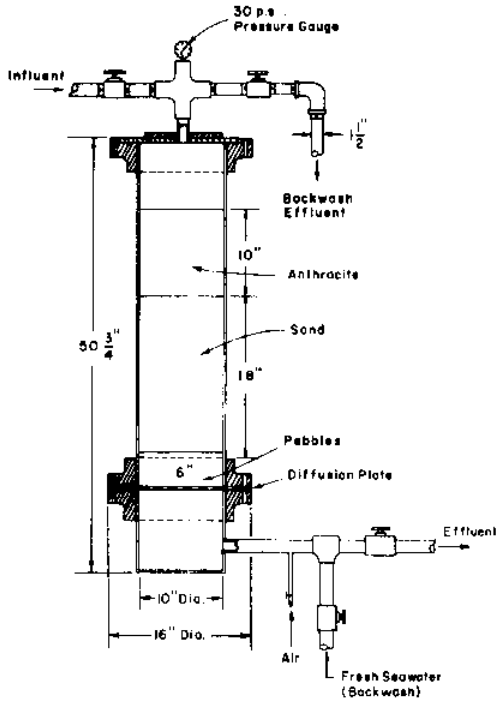

Figure - 4
COLUMN FILTER

Open systems may be used to rear larvae successfully, but are subject to pathogens that may periodically appear in the ocean intake environment and then infect the larvae. Additional filtration and ultra-violet (U.V.) sterilization may be necessary. Several companies produce sterilizers that effectively reduce micro-organisms in sea water. The sterilizers have a quartz sleeve around a 40 watt ultra-violet bulb. Water is circulated in a 3 inch P.V.C. pipe jacket around the sleeve. Approximately 20 liters/min can be sterilized (99% kill) by each 1 bulb unit. The quartz sleeve must be kept clean or the effectiveness of the U.V. treatment will be drastically reduced. Prior filtration with sandfilters or commercial cartridge filters that catch less than 20 micron particles greatly increase the effectiveness of the U.V. unit.

Although a single pass system is the least complex mode of operation, it requires a highly reliable source of clean water. Most locations, even with the required water quality, do not have an ambient water temperature in the range for larval rearing on more than a seasonal basis. Thermal effluent, controlled by proportional mixing apparatus, could provide large quantities of heated water and would be a plentiful and inexpensive water source if other criteria are met. Sand wells produce a source of water that is characteristically much less variable than ambient surface seawater temperature and usually close to the mean annual air temperature. A seawater sand well may offer a reliable water source in semi-tropical locations.

Semi-Closed Systems

In locations where the seawater source is inadequate in terms of quantity, reliability, or quality, a semi-closed system may have distinct advantages

over open or closed systems. A semi-closed mode of operation consists of the kreisels and various components of treatment for water that is reused in the kreisel. Sea water is continuously replaced in the recirculating system but at a rate a fraction of that required by an open system. Since metabolites are diluted and disposed of by replacing water, dependence on biological filtration is avoided. The semi-closed system has some capacity, dependent on metabolite build-up, to continue operation if the water source should fail. Fig. 5 is a representation of an operational system. Temperature control in the semi-closed system is accomplished by a thermostat with a sensor in the sump. The thermostat controls a relay that can activate either inert glass heating elements or a pump that circulates water through a commercial epoxy lined water heater. The same thermostat/pump arrangement can be used with a heat-exchanger. Heating requirements for every situation will have to be calculated by adding heat lost to the air and the difference of the desired culture temperature and the replacement water. The system should then be designed with heating components of sufficient capacity. For example:

A system of 1200 liters was monitored while static and the heat loss to the air was found to be 2°C/hr @ 20°C.

$$1200\ 1\ 2°C\ hr^{-1} = 2400\ kcal/hr$$

The desired replacement rate is 200 l/hr and the temperature of the replacement water is 10°C compared to the culture temperature of 20°C.

$$200\ liter/hr\ (20°-10°)$$

$$= 2000\ kcal/hr$$

The total heat required is:

2000 kcal/hr + 2400 kcal/hr

=4400 kcal/hr

=17450 Btu/hr = 5100 watts

The ratio of replacement water to water recirculating in the system is dependent on the level of treatment given on each pass through the system. The system used at the Bodega Marine Laboratory with sand and anthracite

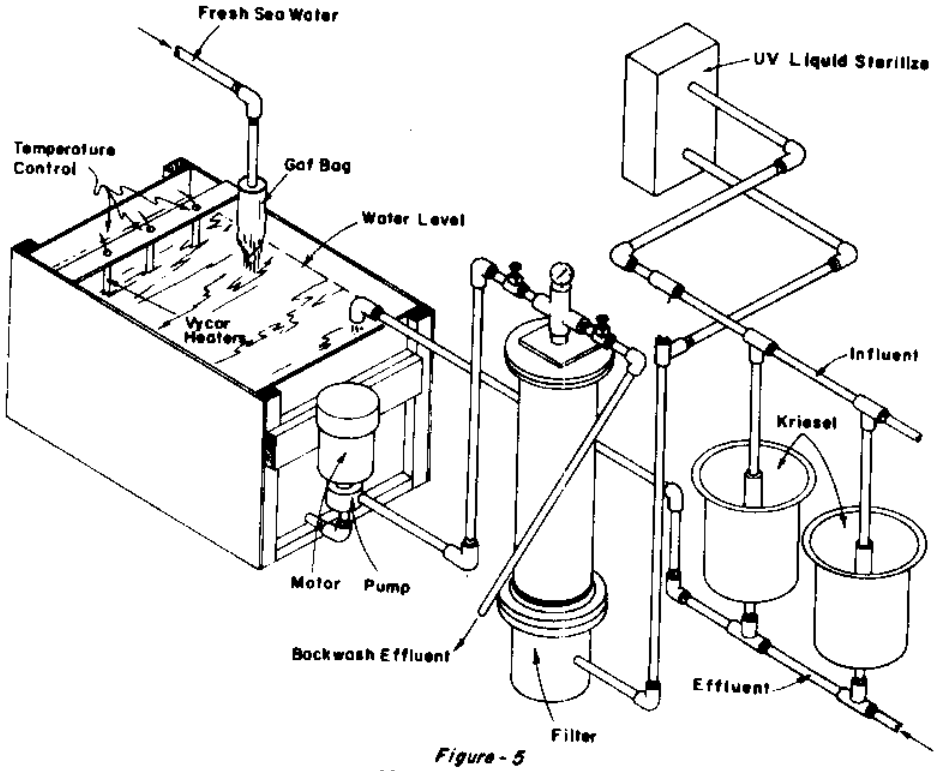

Figure - 5
SEMICLOSED SYSTEM

filtration and U.V. irradiation is operated with a ratio ranging from .08 to .16 depending on load of animals in the kreisels and their feeding rate. With an eight kreisel system of 800 liters, this amounts to 5 to 10 turnovers per day. Each configuration of treatment components must be evaluated using survival as the major criterion.

Closed Systems

The closed system mode of operation has three major advantages: (1) the capability to rear larvae at a site without continually available sea water, (2) the capability, if artificial sea salts are used, to initially exclude pathogens from the system and, (3) reduction in the expense of creating a water source and of pumping and heating replacement water.

Water is recirculated, but without replacement. The build-up of metabolites is the major concern of operating closed systems. This is controlled by a biological filter bed. Ammonia derived from the excretion of the animals and from bacterial decomposition of wasted food is toxic to the larvae. The filter bed is populated with nitrifying bacteria that metabolize the ammonia and convert it to nitrate. Concentrations of nitrate many times higher than that of ammonia are tolerated by the animals, therefore, the water can be used indefinitely if the nitrification process remains stable.

The care of the filter bed is very important to the operation of a closed system. The bed must be preconditioned before use so that the nitrifying bacteria are numerous enough to digest the metabolites before the system is used to rear larvae. The number of animals and feeding level should gradually increase or ammonia production will outstrip the nitrifying ability of the filter bacteria. One conditioning procedure suggested by Spotte (1971) is to hold and feed some hardy animal (e.g. adult lobster) in the system for a week or two to provide an ammonia source for the bed bacteria. Conditioning may take as long as 50 days before the bed reaches its full capacity (Hirayama, 1974).

The system described by Serfling, et al. (1974b) is a closed system (Fig. 6) using a sand substrate filter. This filter, properly conditioned and not overly taxed by nitrogenous waste has been used effectively to

rear larvae. More intensive water treatment has been achieved with a multi-media filter (Fig. 7). The flow is columnar passing through a series of media designed to remove particulate material, reduce ammonia with bacterial action, and remove organics by carbon absorption. U.V. treatment may be added it pathogenic microbes become a source of animal mortality. The columnar configuration of the filter increases the filter media surface and, therefore, allows far more biological filtration per area than does a substrate filter. Several hatches of larvae over periods of up to 6 months have been reared without the addition of sea water. Fresh water is added periodically to make up evaporation losses and maintain salinity at 30 parts per thousand.

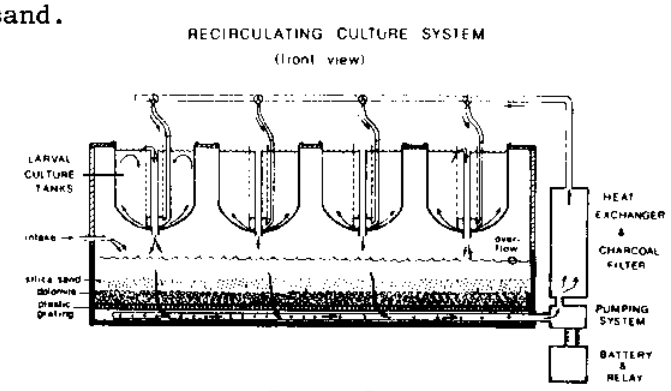

Figure - 6

FRONT, CROSS-SECTIONAL VIEW OF THE RECIRCULATING CULTURE SYSTEM

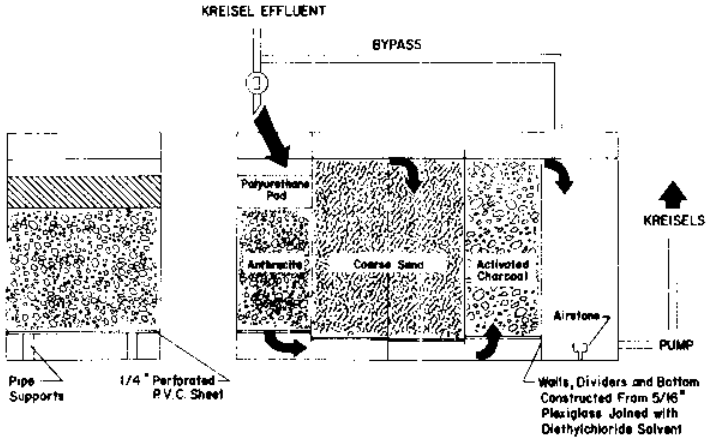

Figure - 7

MULTI MEDIA FILTER, END AND FRONT VIEWS

Summary

Routine rearing of lobster larvae to the juvenile stages has been achieved. Although larvae are susceptible to disease, adequate prophylactic measures can avoid mass mortalities and make the production of post-larval lobsters a relatively reliable process. The rearing kreisel developed at the Massachusetts State Lobster Hatchery has proven to be a near optimum physical habitat for the larval lobster. A number of water systems have demonstrated an ability to provide good rearing conditions if they are designed to control temperature, salinity, fouling organisms, metabolites and infectious disease.

Acknowledgements

This work is in part a result of research sponsored by NOAA, Office of Sea Grant, Department of Commerce, under Grants #04-3-158-22 and #04-5-158-20. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

This work is in part a result of research sponsored by the California State Legislature funds for aquaculture.

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